Haematological Changes Caused by Coccidiosis in Experimentally Infected Broiler Chickens

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ABSTRACT

Haematological alterations caused by coccidiosis in experimentally infected broiler chickens by *Eimeria* spp. The experimental animals (n=100) were randomly allocated into four equal groups, group one (G-I), group two (G-II), group three (G-III) and group four (G-IV) with 25 chickens in each group. The G-I, G-II, and G-III were treatments groups challenged by different *Eimeria* sporulated oocysts, while G-IV served as the control group. In this study, the infective dose of *E. tenella* (G-I), *E. acervulina* (G-II) and mixed *Eimeria* spp (G-III) was 2×10^4 sporulated *Eimeria* oocyst inoculated orally at three weeks of age in broiler chicken and subsequent alterations in different haematological constituents were evaluated at interval of 5, 7 and 9 day of post inoculation. Anaemia caused by *Eimeria* species was characterized by a decreased number of red blood cells and packed cell volume. Statistically highly significant decrease in the total erythrocyte count in all infected groups was recorded compared to control group. The mean value of total erythrocyte count of group one and group two recorded a highly significant decreased on 7 day of post infection. A highly significant increase in total leukocyte count in group one and group two *Eimeria* infected chickens. The statistical analysis of eosinophil and heterophils and lymphocyte showed highly significant increase in group one and group two in comparison to control group. The mean percentage of monocyte in group one, group two and group three revealed a highly significant increase on seven day of post infection.

Keywords: Broiler, Coccidiosis, Haematology

Coccidiosis is a disease of major economic importance in the poultry industry. Confinement rearing and high density flocks of commercial poultry have increased the exposure to coccidiosis. The protozoan parasite of the genus *Eimeria* multiplies in the intestinal tract and causes tissue damage, resulting in the interruption of feeding, digestive processes, nutrient absorption, blood loss and increased susceptibility to other disease pathogens (McDougald and Fitz-Coy, 2008).It is a widespread disease in growing chickens around the world that can seriously restrict the development of poultry production. Nine different species are known; of these, seven *Eimeria* occur in chicken, namely, *E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix, E. praecox* and *E. tenella* (Conway and Mckenzie, 2007).

E. tenella, E. maxima, E. necatrix and *E. acervulina* are the common species which causes coccidiosis in broilers with varying degree of pathogenicity and are dispersed globally.*E. tenella* is the most common and pathogenic coccidia of domestic poultry, which is distributed worldwide. Chickens of all ages are susceptible to coccidiosis, but birds from three to eight weeks of age are the most vulnerable (Yun *et al.,* 2000). Poultry coccidiosis is prevalent in various parts of Ethiopia and affects mostly young growing birds. The increased mortality and reduction productivity in chicken is mainly due to mismanagement, lack of nutritional feeding, parasites both ecto- and endo- parasites diseases are important factors in reducing production and causing loss of chickens (Bereket and Abdu, 2014). The objective of this study was haematological parameter changes in chickens infected with *Eimeria.*

MATERIALS AND METHODS

Study area

The experimental study was conducted in the Faculty of Veterinary Medicine at the premises of Tewodrose Campus, University of Gondar. The area is found in Amhara National Regional State, located in the north-western part of Ethiopia, (12.3° to 13.38° north latitudes and 35.5° to 38.3° east longitudes) (NMA, 2011). The research was conducted from November 2015 to April 2016.

Experimental animals and grouping

One hundred day-old Ross broiler breed chickens were purchased for this experiment from Alema, a private commercial broiler farm, Debre Zeit, Ethiopia. Unsexed day old chickens were randomly and equally allocated into four groups. All groups were maintained at the same management system. The chickens were reared for eight weeks in separate room under strict biosecurity measures and no vaccine was used during the study period. On the start of the experiment the birds were tagged with identification numbers on their wing and leg in each group.

Housing and management of experimental chickens

In this study, day-old chickens were kept with floor housing system. The house, feeder, water utensils were thoroughly cleaned, disinfected prior to stocking of chickens. The utensils were also cleaned daily to avoid reinfection and contamination. Thus, the chickens were reared under strict coccidia free conditions through repeated cleaning and disinfection. Chickens were fed ad libitum on a commercial broiler starter, grower and finisher diet based on their ages throughout the period of the experiment. Continuous heating program with 120 watt bulbs were suspended at head height of the birds. The amount of temperature present in the house was measured and recorded by thermometer and the heat released from brooder was adjusted based on the age of chickens from suspended height. The temperature was maintained at 29-31°C for the first week and was reduced by 1-3°C on weekly basis. Bio-safety of chickens was maintained in study area through fenced farm, protected against wild animals and using footbath. The experimental house had gate and there were special store for feed, disinfectant, personal protective equipment's and closing for the researchers and assistance. The current experiment was conducted with the approval of university of Gondar ethical review board. The experiment was conducted based on the international guidelines of animal experimentation and handling where they were fed adlibitum. The board approved the protocol to be done. Attached here with is the clearance copy.

Experimental design and inoculation of sporulated oocysts

The experimental design used for this research was completely randomized design. The experimental animals (n=100) were randomly allocated into four equal groups: group one (G-I), group two (G-II), group three (G-III) and group four (G-IV) with 25 chickens in each group. The G-I, G-II, and G-III were treatments groups challenged by different *Eimeria* sporulated oocysts, while G-IV served as the control group. All chickens were maintained until the 10th day of age the experiment with a ration containing anticoccidial additives following the recommended producer. After the 10th days of age, the chickens were fed on a ration without anticoccidial additives until the end of experiment. Adlibitum provision of feed and water were maintained. Faecal material from each group was examined at 10th and 21th days of age before the infection, to ensure that the chickens were free from coccidia or other parasitic diseases. Additionally, blood examination was conducted for the detection of pathogenic bacterial agents. One bird from each group was sacrificed and examined to confirm the absence of any parasitic stage of *Eimeria* species and other pathological lesions at 21th day of age.

The treatment groups of chickens (G-I, G-II, G-III) were infected artificially infected with sporulated *Eimeria* oocyts at the age of three weeks as described by You (2014). They were infected orally with infective dose of 2×10^4 sporulated oocysts of *E. tenella*, *E. acervulina* and field isolated mixed *Eimeria* oocysts. The G-I chickens were infected with *E. tenella,* G-II with *E. acervulina* and G-III with mixed *Eimeria* spp. (*E. acervulina, E. tenella, E. necatrix* and *E. maxima*). G-IV was remained as uninfected control groups.

Preparation of infective *Eimeria* species for the experiment

Pure culture *E. tenella, E. acervulina* and mixed identified *Eimeria* species were used for this experiment. The pure cultures of *E. tenella* and *E. acervulina* were acquired from India. The mixed *Eimeria* species were identified from the naturally infected chickens. For identification of these mixed species, oocysts were collected from a total of 22 local and koey koey breed chickens of different sex and ages of clinically coccidiosis suspected chickens were purchased from Gondar town and donated from Kombolcha poultry research and multiplication centre. The chickens were sacrificed in the laboratory at post-mortem room by cervical dislocation using the technique described by Zander (1999). The gastrointestinal tract was grossly examined carefully. Intestinal contents from the respective sections of intestine with lesions were collected. The floatation technique using sodium chloride solution was applied to harvest oocysts (Bowman, 2003).

The harvested *Eimeria* oocysts were identified by a combination of oocyst size, location in the gut and appearance of the lesions (Conway and Mckenzie, 2007; McDougald and Fitz-Coy, 2008). The different species of *Eimeria* were identified according to the length, width and shape index of the individual oocyst after measuring 50 oocysts in each positive sample using a calibrated ocular microscope (McDougald, 2003).

The identified *Eimeria* spp oocysts were spread out in shallow Petri dish containing 2.5% potassium dichromate ($K_2Cr_2O_7$) solution and incubated with a temperature 29ºC, with adequate oxygen and humidity to allowed sporulation as describe by Conway and McKenzie (2007). The sporulation of the oocyst was confirmed by taking a drop of the mixture starting from the second day of incubation and examined for the presence of sporocysts under the microscope. The sporulated oocysts were collected and preserved in 2.5% $K_2Cr_2O_7$ and stored at 4°C. The sporulated oocysts were counted using the McMaster method (Holdsworth *et al*., 2004).

Haematological analysis

A 3 ml sterile syringe and a 23-gauge needle was used to collect blood for evaluation of haematological parameters. Blood samples were collected from brachial vein at 5, 7 and 9 DPI in each group to estimate total erythrocyte and total leucocyte count, differential leucocyte count, haemoglobin concentration and packed cell volume values. The 3 ml whole blood collected from each group was divided into two parts; one part consisted of 1ml of blood sample was transferred immediately into a 3ml sterile tube containing the anticoagulant EDTA for haematological analysis and the rest were allowed to clot to obtain sera for serum enzyme analysis (Samour *et al.,* 2010).

Haemoglobin: Haemoglobin (Hb) value in g/dL was estimated as per the methods described by Benzamin (1985) and Sahli's method was used. The graduated measuring tube was filled with one tenth of normal hydrochloric acid up to graduation mark 2 and placed in the haemometer. After mixing the blood sample, it was drawn up to 20 mark in the pipette. The blood was then transferred into the acid in the measuring tube and the pipette was rinsed by drawing the solution in to it three times. The haemoglobin was converted into brown colour acid haematin within 5 to 10 minutes. After 10 minutes one tenth of normal hydrochloric acid was added drop by drop, mixing the solution with the rod. It was added slowly till the colour matches with the standard on either side of the haemometer. The level of the solution in the tube (upper meniscus) was read and haemoglobin value was expressed as g/dL.

Packed cell volume: The packed cell volume (PCV) in percentage (%) was determined by Hawskey microhematocrit method (Schalm *et al*., 1996). The capillary tubes were filled with blood up to 3/4 of its length. The tubes were sealed at one end with clay and arranged in a special microhaematocrit centrifuge which was fitted with a head for carrying up to 24 capillary tubes. The capillary tubes were arranged in a circular manner with the sealed end outward and the open end towards the centre. The properly covered microhaematocrit centrifuge was set to rotate for 5 min. at 12,000 rpm. The PCV value was read using microhaematocrit reader in percentage.

Total erythrocyte count: The total erythrocyte count (TEC) in millions per cubic millimetre ($10^6/ mm^3$) was calculated by using Hayem's diluting fluid as per the methods described by Coles (1986). Blood sample was initially drawn into the red blood cell pipette up to the 0.5 mark on the stem. Then the diluting fluid was drawn into the pipette up to the 101 mark. The content in the pipette was further

gently mixed for about 2 min. discarding the excess blood was expelled by gently stroking the tip of the pipette on a glass slide and blood was charged in to haemocytometer. The total number of RBCs counted in 5 small squares (4 corners and 1 center) out of 25 small squares and the total number was arrived by multiplying the total number of RBCs by 1000 and result expressed by $10^6/$ mm^3.

Total leukocyte count: The total leucocytes count (TLC) in thousands per cubic millimetres ($10^3/$ mm^3) was counted by standard dilution technique using Thomas fluid as per the method described by Benzamin (1996). Blood sample was drown up to 0.5 mark in to WBCs pipette followed by Thomas diluting fluid up to 11 mark and mixed well. Discarding the excess blood was expelled by gently stroking the tip of the pipette on a glass slide and blood was charged in to haemocytometer. The number of cells was counted in each of the four corner squares of the hemocytometer. The total number of WBCs in 4 corners was multiplied by 50 to arrive at the blood count of WBCs/ mm^3.

Differential cell count: A brachial blood smear was doneat 5, 7 and 9 day of post infection. The smears were stained by Giemsa stain. Stained smear was examined to determine the percentage of each type of leukocyte present. Each white cell was recorded on a differential cell counter, until 100 white cells were counted. The different types of WBC were expressed as percentage.

Statistical analysis

The data obtained were stored in Microsoft excel-2007 and analysed by using STATA version 12. The mean and standard deviation were calculated to describe the haematological parameters recorded during study period. Linear regression analysis was used for the comparisons of haematological parameters in different periods of 5, 7 and 9 DPI in all infected groups were compared with control group. Those differences with P<0.05 were considered statistically significance and those differences with P<0.01 were considered as highly statistical significance.

RESULTS AND DISCUSSION

An experimental trial for *Eimeria* spp. infection in chickens was conducted to evaluate the haematological changes. The haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count and differential leukocyte count that were recorded in all experimental groups on different days viz: 5, 7 and 9 DPI and comparison of these values among different days DPI in different groups using linear regression was summarized in (Table 1).

In all three experimentally infected groups revealed statistically highly significant decrease in haemoglobin (Hb) (P < 0.01) when compared to control group (Table 1). Furthermore, chickens of G-I, G-II and G-III showed highly significant (P < 0.01) reduction in Hb values from 7 to 9 DPI (Table 1). A highly statistically significant (P < 0.01) decrease in packed cell volume level was evident in all the three infected groups compared to control group (Table 1). Also, statistically highly significant (P < 0.01) decrease in PCV values was found on 7DPI in all *Eimeria* infected groups (Table 1). The total erythrocyte count in millions/ mm^3 on 5, 7 and 9 DPI were analysed and recorded. Statistically highly significant (P < 0.01) decrease in the total erythrocyte count in all infected groups was recorded compared to control group (Table 1) The mean value of total erythrocyte count of G-I and G-II recorded a highly significant (P < 0.01) decreased on 7 DPI (Table 1). but in G-I, showed a statistical significant (p<0.05) decreased on 9 DPI.A highly significant (P < 0.01) increase in total leukocyte count in G-I and G-II *Eimeria* infected chickens. However, a statistically significant (p<0.05) increased in GIII were recorded where compared to control group (Table 1). A highly significant (P < 0.01) increase total leukocyte count was observed in G-I and significantly (P < 0.05) increase (p < 0.05) in G-II and G-III on 7 DPI (Table 1).

The mean total differential leukocyte count of heterophils, lymphocytes, eosinophil and monocytes were recorded and analysed at 5, 7 and 9 days after infection in all experimental groups. The statistical analysis of eosinophil and heterophils and lymphocyte showed highly significant (p < 0.01) increase in G-I and G-II in comparison to control group (Table 1). Highly significant (p < 0.01) increased eosinophil in G-I and G-II on 7 DPI (Table 2). The mean percentage of monocyte in G-I, G-II and G-III revealed a highly significant increase (p < 0.01) on 7 DPI, but heterophils values statistical statically (p < 0.05) increased in GII and GIII on 7 DPI (Table 1).

Decreased mean haemoglobin concentration had been recorded in infected groups in comparison with control

Table 1: The linear regression analysis values of Hb, PCV, TEC and TLC values in four groupson 5, 7 and 9 DPI

Groups	DPI	Hb (g/dl)	PCV (%)	TEC (10^6 /mm3)	TLC (10^3 /mm3)
		M±SD	M±SD	M±SD	M±SD
G-I	5	9.8±1.59	20.03±4.97	2.00±0.18	2.99±0.13
	7	1.5±0.40[a]	5.79±1.91[a]	1.20±0.22[a]	4.11±0.24[a]
	9	4.8±1.73[a]	19.03±4.38	1.55±0.35[b]	3.09±0.14
G-II	5	8.75±2.44	15.2±2.40	1.83±0.17	2.71±0.46
	7	1.48±0.43[a]	6.63±0.59[a]	1.35±0.24[a]	3.28±0.19[b]
	9	5.83±1.28[a]	14.46±4.99	1.95±0.13	3.05±0.19
G-III	5	7.09±1.47	20.4±2.2	2.35±0.48	2.99±0.19
	7	4.37±2.64[a]	14.11±4.6[a]	1.85±0.13	3.05±0.08[b]
	9	4.93±1.43[a]	20.06±1.42	2.10±0.29	3.04±0.06
G-IV	5	10.51±1.78	36.05±6.27	2.85±0.24	2.33±0.55
	7	8.29±3.21	39.37±4.96	2.98±0.16	2.61±0.41
	9	9.48±1.54	44.96±10.9	3.05±0.13	2.90±0.91

Note: [a] =highly significant difference (P < 0.01), [b] = significant difference (P < 0.05), g/dl = gram per decilitre, DPI = day of post infection, Ref = reference value, G-I = group one, G-II = group two, G-III = group three, G-IV = group four, Coff. = Coffiecnt, % = percent, mm^3 = millimetre cub, DPI = day of post inception.

Table 2: The linear regression analysis of DLC values in different groups with different DPI

Group	DPI	Heterophils (%)	Monocytes (%)	Eosinophils (%)	Lymphocytes (%)
		M±SD	M±SD	M±SD	M±SD
G-I	5	35.58±4.59	7.63±0.96	8.56±1.33	7.63±0.96
	7	43.85±5.07[a]	9.04±1.44[a]	9.3±1.71[a]	9.04±1.44
	9	39.98±5.59	7.76±1.83	10.71±2.11	7.76±1.83
G-II	5	37.86±6.38	5.9±0.56	7.55±0.73	6.91±1.35
	7	37.86±3.39[b]	8.5±3.24[a]	11.73±1.05[a]	8.50±3.24
	9	35.44±4.07	6.91±1.35	9.33±1.00[a]	5.90±0.56
G-III	5	32.34±6.27	4.75±1.49	4.56±1.68	4.75±1.49
	7	41.36±4.58[b]	6.23±1.47[a]	11.54±5.36	6.23±1.47
	9	36.55±5.81	5±0.866	6.00±2.33	5.00±0.87
G-IV	5	31.91±5.49	5.38±0.92	4.09±1.40	5.38±0.92
	7	32.75±4.59	5.75±1.83	5.89±3.66	5.75±0.83
	9	32.43±12.87	5.56±1.59[a]	6.00±1.85	5.56±1.59

Note: DLC= deferential leukocyte count

group. Thesis findings are in accordance with Ogbe *et al.* (2010) who reported a decrease in the Hb counts in *E. tenella* infected chickens. On contrary, Meskerem *et al.* (2013) who reported that non-significant difference of Hb value in *E. tenella* and *E. brunetti* infected chickens compared with normal value. In comparison with Razzaq *et al.* (2003) demonstrated the lowest Hb and total erythrocyte count (TEC) in quail chicks experimentally infected by *E. tenella*. A significant decrease in the mean PCV values is observe in infected groups when compared with control groups and on 7 DPI in all infected groups showed significant. This could be attributed to the

reduction of blood components due to haemorrhage caused by the disease. Similar records of PCV have been recorded by Wakenell (2010) and Meskerem *et al.* (2013) who reported lower values of PCV in chickens infected with *E. tenella* and *E. acervulina* in comparison to the normal reference values of the chicken. Similarly the result agreed with Turk (2005) who recorded that PCV value reduction from 5 to 10 day in *E. necatrix* infected chickens. A significant decrease in the mean total erythrocyte count in all infected groups particularly on 7DPI was observed in the present study. Similar observations were reported by Irizaary-Rovira (2004) and Patra *et al.* (2010) with marked reduction of TEC values in chickens infected by *E. tenella* and *E. brunetti*. The reduction of TEC on 7 DPI in the infected groups might be due to the severe bleeding and tissue damage in the mucosal surface of intestine occurred at acute stage of infection from the invasion of different *Eimeria* spp. and liberation of large quantity of histamine as a result of due to injury of tissues which caused increased permeability of capillaries and venules allowing exudation of large quantities of fluid. Significant increase in the mean total leukocyte count was observed in the present study with infected groups in comparison with control group. A significant increase was recorded on 7 DPI in GI and GII. Similar findings are reported by Patra *et al*. (2010) and Mohammed (2012) who reported that TLC significant increase in blood of chickens of coccidia infected groups compared with control group. On the contrary, Zulpo *et al.* (2007) reported non-significant difference in TLC when the infected groups are compared with control group. The elevation of TLC couldbe due to the increase of polymorph nuclei number (heterophils and eosinophils) at acute stage of infection. The heterophils infiltration increase immediately after any infection as a first line of defence followed by increase in eosinophil concentration as a response to parasitic infection (Wakenell, 2010).

In the present study, the DLC in chickens infected with *E.tenella,E. acervulina* and mixed isolated *Eimeria* species showed increased lymphocytes, monocytes, eosinophils and heterophils count. These findings are in agreement with Kogut *et al*. (2005) and Meskerem *et al*. (2013) who recorded increased differential leukocyte count of coccidia infected broiler chicken. The increased lymphocyte count might be due to induction of immune response in the infected birds due to increased lymphopoiesis as first step of defence mechanism to infection. On the contrary,

Zulpo *et al*. (2007) who reported that eosinophils and heterophils count were decreased in *E. tenella* infected groups compared with the control group. In the present study, the increment in the number of the leukocytes could be due to the response to infection with coccidiain chicken and severe tissue destruction.

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